Propagation of *Triphyophyllum peltatum* (Dioncophyllaceae) and Observations on its Carnivory

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Abstract

The temporarily carnivorous plant species *Triphyophyllum peltatum* is currently being cultivated at the Botanical Gardens of the Universities of Bonn and Würzburg. In both gardens, it developed carnivorous glandular leaves. This paper complements earlier communications concerning propagation and greenhouse cultivation of this rare West African plant species. We report on our progress with *ex vitro* progeny, on the cultivation of young plants of *Triphyophyllum peltatum,* and on observations on the carnivorous stage.

Introduction

The tropical liana *Triphyophyllum peltatum* is endemic to the rainforests of Western Africa. In its juvenile phase, it runs through a remarkable carnivorous stage as one part of a complex development. The plant is under-represented in private and scientific collections of carnivorous plants. The main reason is the limited availability of viable seeds or living plant material, combined with the difficult reproduction and cultivation. Thus, formation of carnivorous glandular leaves is not frequently observed in greenhouses. Furthermore, although this interesting plant has been known for 80 years, the glandular leaves have rarely been seen in nature due to its scarce occurrence and the difficult political situation in its native countries (McPherson 2008).

Discovery and Taxonomic History of *Triphyophyllum peltatum*

The plant, initially named *Dioncophyllum peltatum*, was discovered in 1928 (Hutchinson & Dalziel). It was first described as the third species of the genus *Dioncophyllum*, which had been known since 1890 (Baillon), but its taxonomic affiliation had always been doubtful. First it was supposedly related to the families Bixaceae and Passifloraceae or considered as a link between these two families (Baillon 1890). Later, *Dioncophyllum* was classified as a genus of the plant family Flacourtiaceae (Warburg 1895; Gilg 1908). A relationship to the carnivorous family Nepenthaceae was also dis-

Figure 1: Shoots of *Triphyophyllum peltatum* cultivated in vitro on Anderson medium. Photo by A. Irmer.

cussed (Sprague 1916; Hallier 1921). In the middle of the 20th century, Airy Shaw reviewed the genus *Dioncophyllum.* He described each of the three known species as independent monotypic genera (*Dioncophyllum tholonii*, *Habropetalum dawei*, and *Triphyophyllum peltatum*) and combined them into the newly generated family Dioncophyllaceae (Airy Shaw 1951). Today, this family belongs to the order Caryophyllales, which includes the related family Ancistrocladaceae and the likewise carnivorous families Nepenthaceae, Droseraceae, and Drosophyllaceae (Barthlott *et al.* 2007; Heubl *et al.* 2006).

The name *Triphyophyllum* refers to the complex life cycle of the liana, which is unique in the plant kingdom: three different types of leaves are produced consecutively during the development of *Triphyophyllum.* Although being a liana as an adult plant, it first develops lanceolate leaves on the condensed stem, followed by the occurrence of additional sticky glandular leaves that possess carnivorous properties. Finally the third type of leaves is formed, characterized by terminal hooks at the tip of the leaf, allowing the plant to climb up trees and shrubs of the rainforest (Porembski & Barthlott 2002). The seeds of *Triphyophyllum* constitute another fascinating feature, being the only seeds in the plant kingdom that are larger than the fruit (so-called secondary gymnospermy – Bringmann & Rischer 2001). The second part of the species name '*peltatum*' (Latin: pelta, Greek: pélte – πέλτη – = shield), refers to the discus-shaped seeds.

Triphyophyllum does not only possess remarkable botanical features, but is also interesting for its chemical constituents. It produces naphthylisoquinoline alkaloids (Bringmann & Pokorny 1995; Bringmann *et al.* 1998, 2003). These structurally, biosynthetically, and pharmacologically outstanding secondary metabolites exhibit promising bioactivities against the pathogens of several infectious diseases. As an example, the alkaloid dioncophylline C shows the potential to cure malaria-infected mice (Bringmann *et al.* 1992; François *et al.* 1997a, 1997b). More recently, other specific activities have been found, *e.g.*, against *Leischmania* parasites (Ponte-Sucre *et al.* 2007). Such naphthylisoquinoline alkaloids are only known from the three species of the Dioncophyllaceae family and in plants of the closely related, likewise small family Ancistrocladaceae.

In this paper we report on our progress in *ex vitro* progeny, on the cultivation of young plants of *Triphyophyllum peltatum,* and on observations on the carnivorous stage.

The Progeny of *Triphyophyllum* - from Axenic Cultures to Young Plants in the Greenhouse

The close cooperation of the research group in Würzburg with scientists from the Ivory Coast (in particular with Prof. L. Aké Assi) provided the Botanical Garden of the University of Würzburg with viable seeds of *Triphyophyllum peltatum*, *i.a.* in 1997. Most of the seeds were sown under greenhouse conditions, yielding plants that grew well for years in Würzburg (Bringmann *et al.* 2002). One of these plants was donated to the Botanical Gardens of Bonn.

In order to achieve axenic cell cultures for phytochemical studies and biosynthetic investigations on the naphthylisoquinoline alkaloids, some of the seeds from the Ivory Coast were surface-sterilized and sown under aseptic conditions on an artificial medium (Bringmann *et al.* 1999). From the sterile seedling, *in vitro* organ cultures (Bringmann *et al.* 1999) of *Triphyophyllum* (shoot cultures, see Figure 1) were successfully established. These sterile shoots were used as the parent material for inducing cell cultures (Bringmann *et al.* 2000), likewise opening the possibility for clonal propagation of this rare plant *in vitro*.

Figure 2: Sterile culture of newly rooted *Triphyophyllum peltatum*. Photo by A. Irmer.

The shoots grown aseptically appeared rootless, which prevented cultivation outside the flasks under greenhouse conditions. In order to generate rooted plants from the cultivated shoots, different phytohormone combinations – as usually used for root induction – were added to the medium. Unfortunately, this approach did not deliver completely satisfying results. Among the methods tested, the transfer of the shoots into sterile water was the most efficient one; under these conditions, at least 25% of the shoots showed good root growth (Bringmann & Rischer 2001).

Spontaneous rooting was occasionally observed during routine subcultivation of the shoots, especially if the shoots, instead of being transferred to fresh medium after four weeks, as usual, were kept on depleted medium for an extended time exceeding the usual four-week subculture interval.

Aseptic rooted plants were transferred to sterile Seramis® (Mars GmbH, Verden) substrate (see Figure 2) soaked with a solution of one-fifth strength MS macro elements (Murashige & Skoog 1962). The rootless shoots were transferred to fresh Anderson medium (Anderson 1980) to continue the sterile organ culture.

The small (and still sterile) plants were cultivated in Seramis® substrate for several weeks under the same growth conditions as previously applied to the shoots (Bringmann & Rischer 2001) so that they continued the root and shoot growth / development.

For the acclimatization to greenhouse conditions, which was successfully accomplished 3-4 months later, the plants were transferred into small pots filled with Seramis® and grown at 95% humidity and 25°C on a heated bed with constant light between 8 h and 20 h per day, if necessary using artificial daylight (Bringmann *et al.* 1999).

The plants in the greenhouse were watered daily with rainwater and in the first weeks without any fertilizer to avoid salt stress. During this time only some small leaves (max. 4 cm long) were produced. After 2–3 months in the greenhouse, a release fertilizer – 0.5 g MannaCote M6 (Wilhelm Haug GmbH & Co KG, Ammerbach) per pot – was added to improve nutrient supply of the young plants, which resulted in a slightly increased leaf size (*ca.* 5 cm long). The growth during summer time was very slow, whereas from the beginning of fall on, the growth rate and leaf size increased drastically (now up to 10-cm long leaves).

Figure 3: Different stages of the reverse circinate unfolding of a carnivorous organ of *Triphyophyllum peltatum* with first mature glands. The leaf-like part at the base is not developed at this stage. Photo by A. Irmer.

Figure 4: The development of the leaf base of the carnivorous organ of *Triphyophyllum peltatum*. Photo left by A. Irmer; right by K. Rembold.

The method presented here permits production of rooted young plants of *Triphyophyllum peltatum* for cultivation in a greenhouse and without loss of plant material: Non-rooted shoots were not lost but could be further cultivated under sterile conditions.

Formation of Glandular Leaves

The *Triphyophyllum* plant in the Botanical Gardens of Bonn developed three carnivorous leaves in July 2006 (see Front Cover). In December 2007, one of the previously sterile young plants in Würzburg likewise started developing two carnivorous glandular leaves followed by a period of rapid growth, leaf enlargement and, surprisingly, formation of more carnivorous organs after 6 months (up to five on one plant). In May 2008, two more plants at the Botanical Garden of Würzburg formed glandular leaves, too.

All glandular leaves observed at the Botanical Gardens of Bonn and Würzburg showed the typical reversely circinate unfolding (Green *et al.* 1979), similar to that of *Drosophyllum* (see Figure 3). During this unfolding process, all three types of glands – big and stalked, small and stalked, and sessile (Green *et al.* 1979) – were developed in parallel on the extended midrib. Each carnivorous organ developed a broadened leaf base (see Figure 4), while the midrib was unfolding.

The size of the broadened leaf base differed from organ to organ, ranging between 2 and 8 cm in length and 0.5 and nearly 2 cm in width. The green leaf base and the glandular parts showed a reciprocal size ratio: the bigger the leaf base, the smaller the glandular part. The tip of fully unfolded and mature carnivorous organs was formed by a stalked gland, *ca.* 1.5 to 2 times bigger than the size of other glands (see Figure 5). In some cases, the tip was bifurcated looking like a two-toothed fork. Such a modification had already been observed earlier (Schmid 1964). It is reminiscent of the subsequently formed hooked leaves of *Triphyophyllum*. The mature carnivorous organs remained erect and survived 3–4 weeks, and then they died-off rapidly and were shed immediately.

From the first fully developed gland on, the carnivorous organs secreted mucilage and captured small insects – like sciarids and chironomids – or spiders. The mucilage is known to contain digestive enzymes typical of carnivorous plants (Green *et al.* 1979; Bringmann & Rischer 2001); and it had been shown that the glandular leaves actively take up the amino acid L-alanine (Bringmann *et al.* 1996). The carnivorous glandular leaves of *Triphyophyllum* are considered as passive flypaper

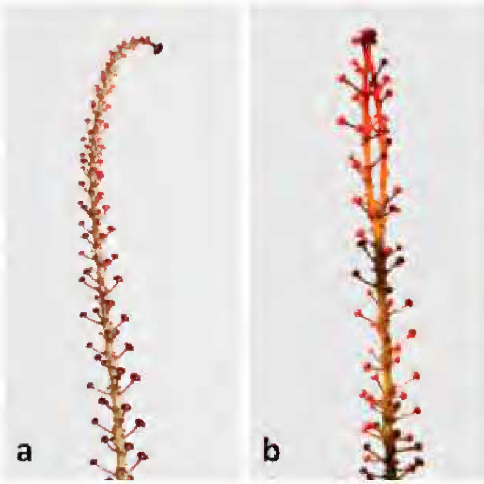

Figure 5: The apices of glandular leaves of *Triphyophyllum peltatum* showing the big tip gland (a) and a bifurcated tip (b). Photo (a) by A. Irmer; (b) by K. Rembold.

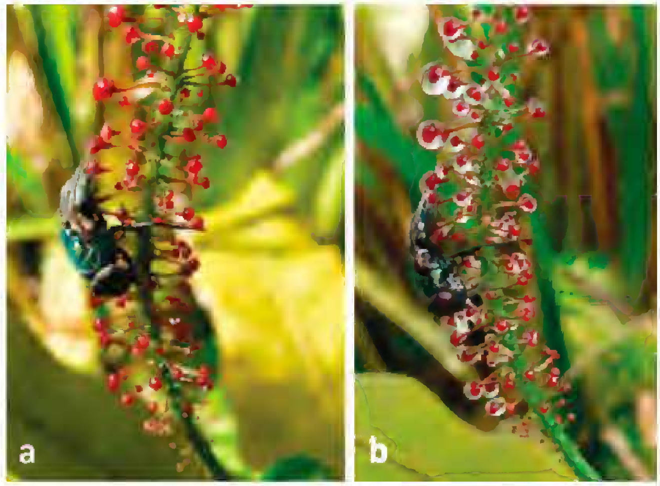

Figure 6: Glandular leaf of *Triphyophyllum peltatum*. On July 17, 2006, 6:00 pm: glands with little mucilage (a). On July 18, 2006, 9:30 am: glands were covered abundantly with secretion (b). Photos by K. Rembold.

traps (Marburger 1974; Green *et al.* 1979; Marburger 1979). Although the carnivory of *Triphyophyllum* is proven, but it has never been investigated experimentally whether the stalked glands show any movement when stimulated by prey.

The Flypaper Traps of *Triphyophyllum* are Passive

A blowfly (Calliphoridae) was placed alive on a two-week old glandular leaf of the *Triphyophyllum* specimen in the Botanical Gardens of Bonn in order to test whether the glandular organs of *T. peltatum* are passive flypaper traps or if they are actively capable of tentacle or leaf movements, and to test the trap efficiency. The insect adhered to the mucilage secreted by the stalked glands but was able to escape after *ca.* 30 minutes. The successful escape of the fly suggests that the natural prey spectrum of *T. peltatum* might consist of smaller or weaker animals (Green *et al.* 1979; Bringmann *et al.* 2001; McPherson 2008). The fly was incapable of flying because it was covered in mucilage and remained trapped after a second placement on the leaf.

The carnivorous organ with the prey was constantly monitored and photos were taken in intervals during July 17 and 18, 2006. During this time, tentacle or leaf movements were not noticed. Even during the following days neither the leaf lamina nor the tentacles changed their orientation. This confirms that the glandular leaves of *Triphyophyllum peltatum* act as passive flypaper traps. A discoloration of the stalked glands after contact with the prey, as reported by Marburger (1979), was not observed.

The amount of secreted mucilage however, was notably altered in the course of the days (see Figure 6), and decreased towards the evening. In the mornings, some drops on adjacent tentacles were found to have converged as a consequence of increased secretion.

Interestingly, *Triphyophyllum peltatum* produced again lanceolate leaves, both in the Botanical Gardens of Bonn and Würzburg after the carnivorous phase. Obviously, the succession of the juvenile stage followed by a carnivorous phase prior to the development of the adult liana is not obligatory. It had been observed earlier that *T. peltatum* can also progress from the juvenile directly to the adult liana stage without forming any carnivorous organs (Bringmann *et al.* 2002). Adult plants in nature are able to produce carnivorous leaves on climbing stems (McPherson 2008).

In summary it can be stated that *Triphyophyllum* shows remarkable flexibility in terms of its morphological development, the determining factors of which still remain elusive.

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